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Rate of Small Intestinal Cell Renewal and Fecal Excretion of Steroids in Rats Fed Soybean Protein or Casein

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In rats acclimatized to meal-feeding, soybean protein relative to casein stimulated fecal steroid excretion, in particular acidic steroids in a few day. The parameters of mitotic activities of the epithelial cells of the small intestine, in terms of mitotic figures per a crypt and crypts having mitotic figures per total crypts, were both similar to or slightly higher in rats fed soybean protein than in those fed casein. The results thus suggests that the luminal event rather than the rate of cell renewal is responsible for an instant increase in fecal steroid excretion due to feeding soybean protein.

INTRODUCTION

The hypocholesterolemic effect of soybean protein, in relation to casein, has long been recognized in humans (Sirtori *et al.*, 1977 ; Carroll *et al.*, 1978 ; Wolfe *et al., 1981)* and in experimental animals (Carroll and Hamilton, 1975 ; Kritchevsky *et al.*, 1979 ; Nagata *et al.*, 1981 ; Beynen *et al.*, 1983). The possible trigger responsible for the reduction of the serum cholesterol level is an increased fecal steroid excretion (Carroll, 1981 ; Sugano, 1983). It is generally accepted that cholesterol excreted in the intestinal lumen as bile juice is a major source of fecal neutral steroids when the animals were fed diets free of cholesterol. The intestinal eqithelia desquamated into the lumen also contribute to the fecal neutral steroids, though the amounts presumably varies depending on the physiological state of the host animals (Lutton, 1976). However, the renewal of the small intestinal epithelium during feeding different types of protein remains obscure. On the other hand, the recent studies indicate the interference of the partial digests or hydrophobic peptide fragments of soybean protein with reabsorption of bile acids (Yashiro *et al.*, 1985 ; Iwami *et al.*, 1986), thus suggesting an immediate increase in the fecal acidic steroids on feeding soybean protein. The interfering potential of the corresponding casein hydrolysates appear to be considerably low.

In the present study we investigated the effects of soybean protein on the renewal of the small intestinal cells and the fecal excretion of neutral and acidic steroids of rats during the transitional periods after replacing the non-purified diet by the cholesterol-free purified diet containing either soybean protein or casein.

MATERIALS AND METHODS

Animals and Diets

Male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka) weighing

approximately 150 g were meal-fed (1000 to 1200 hours) a commercial non-purified diet (type NMF, Oriental Yeast Co., Tokyo) for two weeks after arrival. The diet was then replaced by experimental diets composed in percent of protein 20, corn oil 5, mineral mixture 4, vitamin mixture 1, choline chloride 0.2, cellulose 2, and sucrose to 100. Either isolated soybean protein (Fujipro R, Fuji Oil Co., Osaka) or casein (Wako Pure Chemicals Industries, Osaka) was used as a source of protein. Mineral and vitamin mixtures according to Harper (1959) were purchased from Oriental Yeast Co. The meal-feeding was adopted in order to drive a synchronized mitosis in the small intestinal epithelium (Potten *et al.*, 1977). At timed intervals, rats, three per group except otherwise mentioned, were killed at 1300 hours under ether anesthesia, blood was collected from the abdominal aorta and immediately excised the small intestine. A portion of the duodenum, jejunum and ileum was cut open, spread on a piece of cardboard, fixed in 10 % formalin in phosphate buffer pH 7.0 for 12-24 hours and then processed by the paraffin technique. The sections were cut longitudinally at 4-5 μm and stained with hematoxylin-eosin. Feces were collected daily and lyophilized.

Mitotic activities

Mitotic activities were measured according to Altmann (1976). Percentages of mitotic figures per crypt (M/C) and crypts having mitotic figures per total crypts (MC/TC) were calculated. Each mitotic figure from prophase to telophase was counted, but early prophase and late telophase were excluded from counting. Since the number of mitoses varied from crypts to crypts, well oriented thirty to fifty crypts were measured in each histological section for calculation of M/C. Microscopic examinations were carried at 300-600 magnification.

Steroid Analyses

Fecal steroids were analyzed by gas-liquid chromatography (GLC) on the extracts obtained according to the method of Uchida *et al. (1977).* Acidic steroids were assayed as methyl ester-acetate derivatives using a glass column (3 mm *x* 2 m) packed with 3 % AN-600 on Gas Chrom Q (Gaschro Koggyo Inc., Tokyo) with 23-nor-5β-cholanic acid -3α,12α-diol (Steraloids, Inc., Wilton NH) as a standard (Kuriyama *et al., 1979).* Neutral steroids were assayed as trimethylsilyl ether using a glass column (3 mm X 2 m) packed with 3% OV-17 on Gas Chrom Q (Gaschro Koggyo Inc., Tokyo) with 5α-cholestane as a standard (Sugano *et al., 1983).* Serum cholesterol was measured by the enzymatic method (Cholesterol C-Test, Wako Pure Chemicals Industries, Osaka).

Statistical analysis

Data were analyzed by the Student's t-test.

RESULTS

Growth parameters and serum cholesterol levels

Table 1 shows the body weight gain, food intake and serum cholesterol concentration. Dietary protein-dependent differences in the serum cholesterol levels were found only at the third day. Body weight gain at the tenth day was slightly low in rats fed soybean protein regardless of the similarity of the amount of the diet consumed.

Table 1. Effects of dietary proteins on growth, food intake and serum cholesterol concentration.

Groups	Body weight gain (g)	Food intake (g/day)	Serum cholesterol (mg/dl)
1st day			
Casein	–	8±1	70.2± 9.8
Soybean	–	8±1	52.4±10.3
3rd day			
Casein	−1±2	.2±1	70.7±3.2
Soybean	Oil	8±1*	53.8±4.4*
5th day			
Casein	7±3	11±2	82.7±3.9
Soybean	10±2	11±3	79.4±4.6
10th day			
Casein	28±9	1512	84.7±10.7
Soybean	20	16	87.0

Each value represents mean±SE of 3 rats, except for 2 rats for the 10th day soybean group. *Significantly different from the casein group at $p < 0.05$.

Fecal steroid excretion

As shown in Table 2, at the third day, weight of feces of the soybean protein group was significantly higher than that of the casein group, but the difference disappeared thereafter. Daily fecal excretion of neutral steroids tended to be higher in the soybean protein group throughout the feeding periods. The extent of microbial transformation of cholesterol to coprostanol was in general comparable between the two groups. In contrast, fecal acidic steroid excretion was significantly high in rats fed soybean protein ; the excretion increased gradually with the lapse of the experimental periods in the soybean protein group, while it was apparently unchanged in the casein group.

Table2. Effects of dietary proteins on fecal steroids excretion.

Groups	Weight of feces (g/day)	Coprostanol	Neutral steroids Cholesterol (mg/day)	Total	Acidic steroids (mg/day)
3rd day					
Casein	0.33±0.07	1.63i0.72	1.24±0.31	2.87f0.56	2.80±0.54
Soybean	0.53±0.04*	3.12f0.61	1.45i0.21	4.57±0.82	7.82±2.10*
5th day					
Casein	0.44f0.06	2.26±0.23	1.26f0.14	3.52±0.31	4.24f0.60
Soybean	0.59f0.11	2.20±0.96	2.65f0.79	4.85±0.90	10.9±2.14*
10th day					
Casein	0.71±0.10	3.44±0.53	.23±0.19	5.67±0.51	3.97±0.90
Soybean	0.60	3.49	1.80	5.29	14.3

Each value represents mean±SE of 3 rats, except for 2 rats for the 10th day soybean group. *Significantly different from the casein group at $p < 0.05$.

Mitotic parameters

As shown in Table 3, mitotic activities in terms of mitoses per a crypt (M/C) and

crypts having mitotic figures per total crypts (MC/TC) were both virtually the same between the two groups, although they were significantly higher in the soybean protein group in some occasions.

Table 3. Effects of dietary proteins on mitotic activities of small intestinal epithelia.

	Duodenum		Jejunum		Ileum	
	M/C	MC/TC (%)	M/C	MC/TC (%)	M/C	MC/TC (%)
3rd day						
Casein	5.2±0.3	23.7±3.5	4.9±0.2	26.1±4.7	5.2f0.4	17.4±3.6
Soybean	5.5±0.4	30.3±1.2*	5.0f0.4	28.5f0.7	5.0±0.3	22.5±2.4*
10th day						
Casein	5.1±0.1	33.0±2.3	4.8f0.8	24.3f0.6	4.9±0.5	26.1f3.0
Soybean	5.6	32.0	5.3	25.9	5.0	26.9

Each value represents mean±SE of 3 rats, except for 2 rats for the 10th day soybean protein group.
M/C : mitotic figures per a crypt,
MC/TC: crypts having mitotic figures per total crypts.
*Significantly different from the casein group at $p < 0.05$.

DISCUSSION

In the present study a marked increase in fecal acidic steroids was observed shortly after meal-feeding soybean protein as compared with casein. The effect of soybean protein on the fecal neutral steroids was rather moderate and was insignificant throughout. The serum cholesterol level of the soybean protein group was, however, not necessarily lower than that of the casein group through the experimental period in contrast to the case of the ad libitum feeding (Nagata *et al.*, 1981). Tanaka *et al.* (1984) also demonstrated a significant increase in fecal acidic as well as neutral steroids in rats fed cholesterol-free diets containing soybean protein for 3 weeks as compared with those fed casein. It is anyhow plausible in these studies that an increase in fecal bile acid excretion is the initial event to produce the reduction of the serum cholesterol level. As we fed cholesterol-free diets to rats, fecal neutral steroids almost exclusively originate in the bile and the desquamated intestinal epithelial cells. Thus, the present observation suggests that the bile excretion and intestinal cell renewal are not largely influenced by the type of dietary protein. Regarding the marked stimulation of fecal acidic steroid excretion, it seemed likely that the partially digested soybean protein, in preference to the casein digests, could adsorb biliary bile acids and interfere with the entero-hepatic circulation, since significantly more soluble nitrogen remained in the proximal intestine in rats fed soybean protein than in those fed casein (Yashiro *et al., 1985).*

The mitotic activity is known to be influenced by a variety of dietary factors including energy and protein intake, and body weight gain or loss (Hopper *et al., 1972* ; Koga and Kimura 1980). Although daily food intake as meal-feeding was lower than

that as ad libitum feeding, mitotic activities were seemed to be apparently uninfluenced by the restricted meal and body weight gain during such a short period as we employed here. The number of mitotic figures per a crypt in the present experiment was comparable with that reported by Potten ***et al.*** (1977), approximately 4.8 %. Duodenal and ileal mitotic activities (MC/TC) of the soybean protein group at the third day were higher than those of the casein group regardless of a significantly low food intake, suggesting a slight hypertrophic effect of soybean protein.

The bile acids constitute important regulatory factors influencing enterocyte proliferation, migration and loss (Roy et al., 1975). As Tanaka ***et al.*** (1984) observed the similarity of the concentration and output of biliary bile acid between rats fed soybean protein and casein, it seems likely that the hypertrophic effect of soybean protein, if any, is presumably not synchronized with the increased excretion of the bile acid into the intestinal lumen.

We conclude that the initial response to soybean protein ingestion is an instant elevation of fecal excretion of steroids particulary bile acids. This will cause dynamic changes in the parameters of cholesterol metabolism such as cholesterol synthesis in the liver and small intestine in order to compensate the increased loss of steroids (Nagata ***et al.,*** 1982).

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